

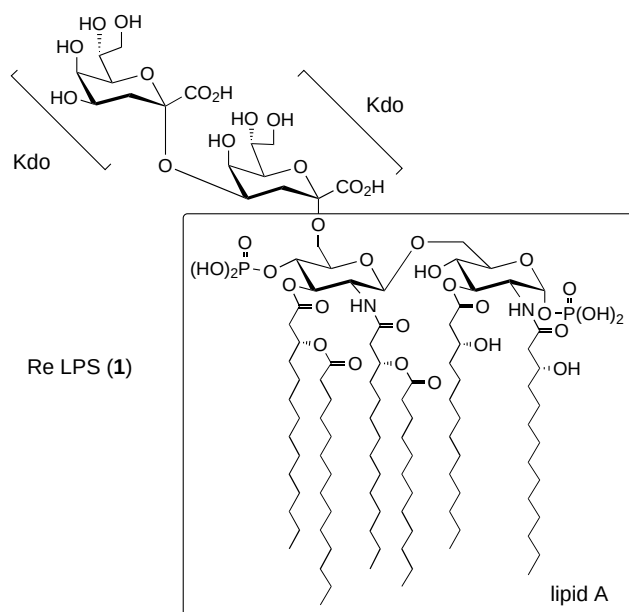
[15] *n* Type: *m/z* calcd: 5445; type I found: 5444 (negative ion ESI-MS); type II found: 5440 (MALDI-MS, matrix: 3-hydroxypicolinic acid/ammonium citrate). *h* Type: *m/z* calcd: 4154; type I found: 4151 (MALDI-MS, matrix: 2,6-dihydroxyacetophenone/ammonium citrate); type II found: 4127 (MALDI-MS, matrix: 2,6-dihydroxyacetophenone/ammonium citrate). *H* Type: *m/z* calcd: 6683; type I found: 6669 (MALDI-MS, matrix: 2,6-dihydroxyacetophenone/ammonium citrate); type II found: 6671 (MALDI-MS, matrix: 3-hydroxypicolinic acid/ammonium citrate).

[16] H.-Y. Li, Y.-L. Qiu, Y. Kishi, *ChemBioChem*, in press.

First Total Synthesis of the Re-Type Lipopolysaccharide**

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Lipopolysaccharides (LPS) are ubiquitous glycoconjugates located on the surface of Gram-negative bacteria such as *Escherichia coli*, and exhibit multiple and potent biological activity, both toxic and beneficial, towards higher animals.^[1] LPS consist of a phosphorylated acyl glucosamine disaccharide covalently bonded to a polysaccharide. The former glycolipid, lipid A, is the entity responsible for most of the biological activity of LPS.^[2, 3] Lipid A is therefore never present on living bacterial cells in the free form; it is an artificial product obtained only by the acid hydrolysis of LPS. The simplest LPS molecule that is known to be present on bacterial cells and that comes into contact with animal cells is the so-called Re-type LPS produced by the *Escherichia coli* Re mutant (Re LPS, **1**).^[4] This LPS derivative is composed of lipid A and two molar equivalents of 3-deoxy-D-manno-2-octurosonic acid (formerly called 2-keto-3-deoxy-D-manno-octonic acid (Kdo)). The chemical synthesis of Re LPS is not only a highly challenging goal, but would also contribute towards the elucidation of the biological and physicochemical role of the sugar moieties linked to lipid A. It has often been implied that the polysaccharide portion enhances or modifies the activity of Re LPS.^[5] In fact, Re LPS that only has two units of Kdo was reported to exhibit more potent antitumor and cytokine-inducing activity than lipid A.^[6, 7] However, these observations have never been confirmed owing to the



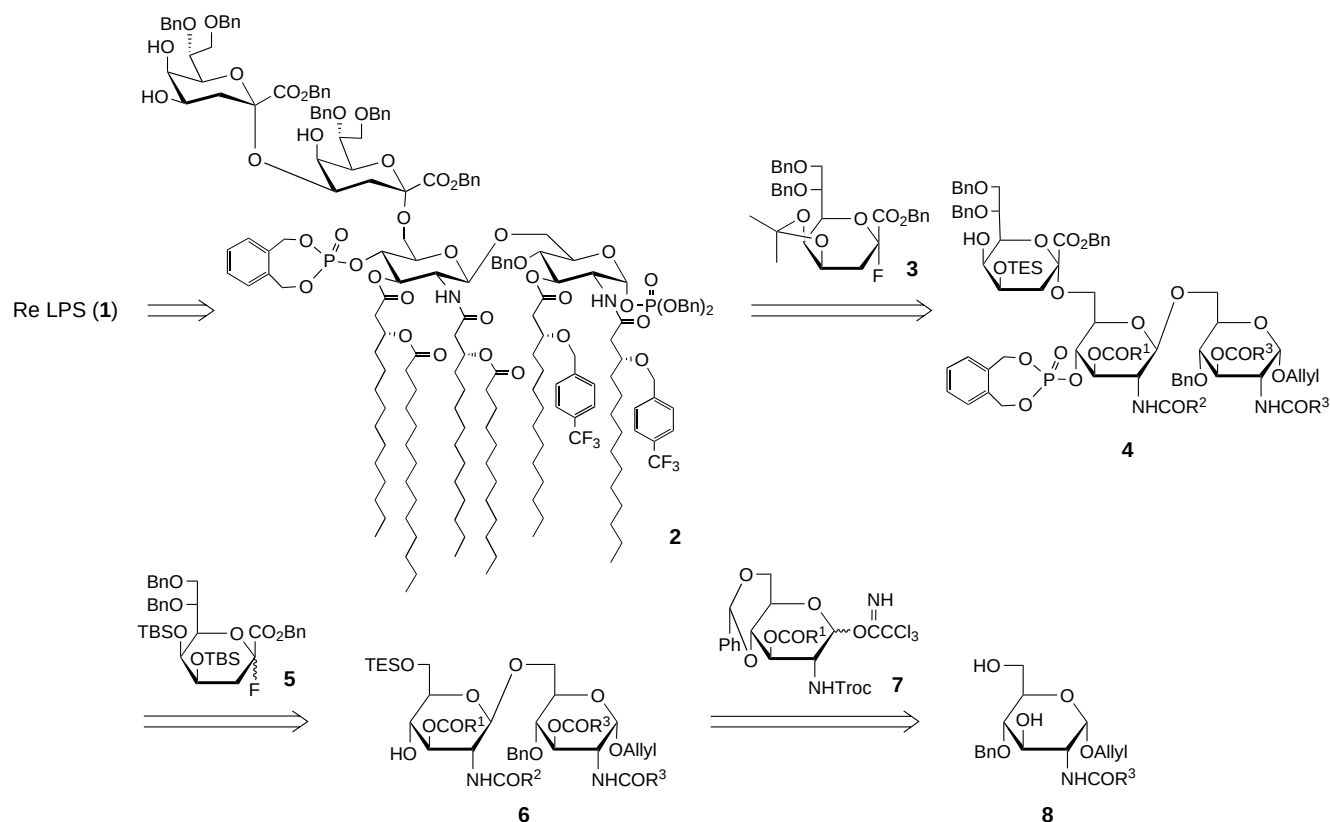
inherent heterogeneity and the difficult purification of natural specimens. An efficient synthesis of Re LPS would also provide easy access to various structural congeners required for the study of structure–activity relationships. Such a strategy based on chemical synthesis has been effective in our studies of the role of acyl and phosphoryl moieties in the bioactivity of lipid A.^[8]

Until now, only partial syntheses of Re LPS have been reported, for example, our synthesis of the 1-*O*-dephospho analogue.^[9] The total synthesis of Re LPS has not yet been accomplished because of the difficulty in synthesizing the complex structure, which contains highly acid-labile glycosyl phosphate, as well as base-labile ester functional groups in the amphiphilic structure. A high-yielding and stereoselective formation of α -Kdo glycosides^[10–13] is a key step towards the synthesis of Re LPS. We have recently completed an efficient synthesis of lipid A^[8c, 14] and its labeled analogues^[15] by means of an optimized synthetic pathway coupled with a high-yielding efficient purification method for the final products based on liquid–liquid partition.^[16] These achievements prompted us to undertake the synthesis of Re LPS (Scheme 1). Herein we describe an efficient stereoselective glycosylation method with Kdo donors, and the first total synthesis of Re LPS. A direct comparison of the biological activities of synthetic and natural specimens is also reported.

Based on our experience with the synthesis of lipid A and its analogues,^[8, 9, 15, 16] we employed benzyl-type groups that are removable by neutral hydrogenolysis, as they are resilient protecting groups for the hydroxy, carboxy, and phosphate groups in **2**. The 4-(trifluoromethyl)benzyl group, which was found to be resistant to oxidation but readily removable by hydrogenolysis,^[17] was used especially for the protection of the hydroxy groups on the 3-hydroxyacyl residues. Unsubstituted benzyl groups at these positions are prone to air-oxidation and gradually are transformed into the corresponding benzoyl groups during storage, even at -5°C .^[17, 18] The tetrasaccharide backbone of **2** was formed by the successive coupling of two

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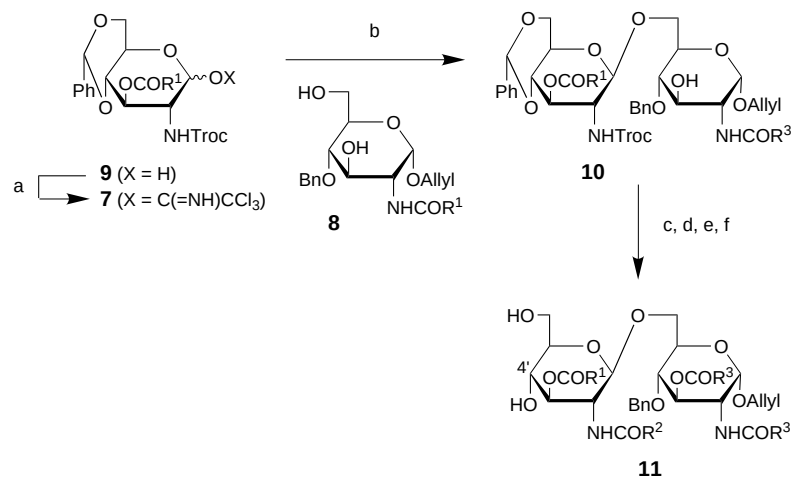


Scheme 1. Retrosynthesis of Re LPS (1). The tetrasaccharide backbone was constructed by sequential glycosylation reactions starting from terminal glucosamine 8. Bn = benzyl, R¹COOH = (*R*)-3-(tetradecanoyloxy)tetradecanoic acid, R²COOH = (*R*)-3-(dodecanoyloxy)tetradecanoic acid, R³COOH = (*R*)-3-(4-(trifluoromethyl)benzyloxy)tetradecanoic acid.

different, suitably protected Kdo donors (3 and 5) to the β -D-glucosamine disaccharide derivative 6, which was prepared by starting from the glycosylation of 8 with 7, using polymer-supported reagents (Scheme 1).

Disaccharide 11, a precursor of 6, was synthesized as shown in Scheme 2. The 3-*O*-acylated glucosamine derivative 9^[8] was

readily prepared in six steps from D-glucosamine, and transformed into glycosyl trichloroacetimidate 7 by using trichloroacetonitrile and Dowex 1-X8 (OH⁻ form) as a polymer-supported basic catalyst. The resin was packed in a MicroKan, so that the work-up was easier than that in conventional methods using either inorganic (e.g. Cs₂CO₃) or organic (e.g.



Scheme 2. Synthesis of lipid A moiety 11. a) Dowex 1-X8 (OH⁻ form) in MicroKan, CCl₃CN, CH₂Cl₂, 3 h (100%); b) Nafion-TMS, MS4A, CH₂Cl₂/perfluorohexane (3/1), 24 h (73%); c) (*R*)-3-(4-(trifluoromethyl)benzyloxy)tetradecanoic acid, DCC, DMAP, CH₂Cl₂, 22 h (85%); d) Zn–Cu, AcOH, 1.5 h; e) (*R*)-3-(dodecanoyloxy)tetradecanoic acid, DCC, CH₂Cl₂, 18 h (85% over two steps); f) TFA, H₂O, CH₂Cl₂, 2 h (90%). DCC = dicyclohexylcarbodiimide, DMAP = 4-(dimethylamino)pyridine, MS4A = molecular sieves (4 Å), TFA = trifluoroacetic acid.

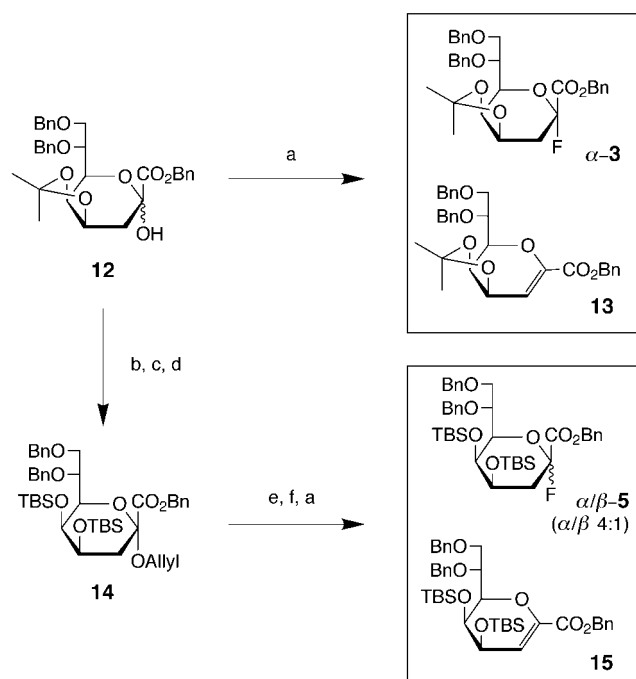
1,8-diazabicyclo[5,4,0]undec-7-ene) bases.^[19] The desired trichloroacetimidate 7 was obtained quantitatively by simply removing the resin-filled MicroKan from the mixture after the reaction had gone to completion, and evaporating the solvent. Glucosamine derivative 8^[8b] was coupled with saccharide 7 by using another polymer-supported catalyst, Nafion-TMS, as a Lewis acid.^[20] This reaction did not proceed well in either CH₂Cl₂ or perfluorohexane, but occurred smoothly in a 3:1 mixture of the two solvents at room temperature over 24 h to give the desired β (1 $\rightarrow$ 6)-disaccharide 10 in 73% yield. This glycosylation is a new method for activating a glycosyl donor.^[21] The hydroxy group of 10 was then acylated with a (trifluoromethyl)benzyl-protected 3-hydroxy fatty acid in 85% yield. Cleavage of the (2,2,2-trichloroethoxy)carbonyl (Troc) group at the 2'-position, followed by N-acylation with (*R*)-3-(dodecanoyloxy)tetradecanoic acid^[22] and removal of the benzylidene group, gave the tetraacylated disaccharide 11 in

77% yield. The reaction of Kdo donors at the 6'-hydroxy group of the lipid A moiety is sluggish in the presence of the 4'-*O*-phosphono group (data not shown), and hence coupling with Kdo was examined prior to 4'-*O*-phosphorylation.

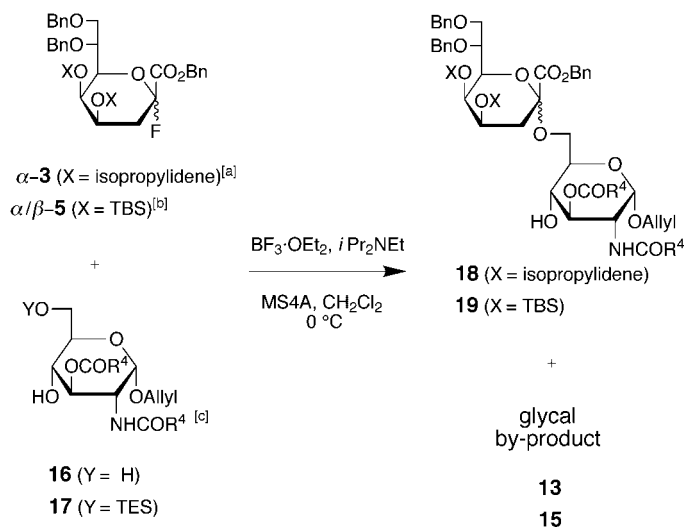
The key α -selective glycosylation step with a Kdo donor is not a well-established reaction and thus was intensively investigated in the present work. The major problem of this glycosylation is the lack of a functional group that controls the anomeric stereoselectivity at the neighboring C3 position of Kdo. We previously obtained a high α -selectivity by using 4,5-*O*-isopropylidene-protected Kdo fluoride as a donor. With this Kdo donor, the undesirable β -side attack by a glycosyl acceptor is prevented by the presence of the bulky isopropylidene group.^[12a] This approach was successfully extended in the present work. Another problem encountered during the glycosylation was the formation of considerable amounts of glycals in side reactions. The undesirable formation of glycals^[23] was drastically suppressed without the loss of α -stereoselectivity by replacing the 4,5-*O*-isopropylidene group with other bulky protecting groups, as described below.

The two Kdo fluorides **3** and **5**, which are protected with isopropylidene and *tert*-butyldimethylsilyl (TBS) groups, respectively, were synthesized from a common precursor **12** (Scheme 3). Compound **12** was prepared from D-mannose in nine steps.^[24] The reaction of **12** with (diethylamino)sulfur trifluoride (DAST) gave an inseparable mixture of α -glycosyl fluoride (α -**3**) and glycal **13** (ca. 3:1 ratio, 88%) which was used directly in the subsequent glycosylation.^[25] For the synthesis of TBS-protected Kdo fluoride **5**, the anomeric position of **12** was temporarily protected as the allyl glycoside in 89% yield.^[26, 27] Replacement of the isopropylidene group with a TBS group, followed by deallylation and fluorination afforded α/β -**5**. Only trace amounts of glycal by-product **15** were formed in the fluorination of the hemiacetal derived from **14**. This suggests that the oxocarbenium ionic intermediate generated from **14** is more stable than the one generated from **12**.

Glycosylations with the Kdo donors α -**3** and α/β -**5** were carried out with 6-hydroxy and 6-*O*-silylated model glucosamine acceptors **16** and **17**^[3] (Scheme 4, Table 1).^[12a, 28] The reactions were carried out with excess $\text{BF}_3 \cdot \text{OEt}_2$ ^[29] in the presence (Table 1, entries 1 and 2) or absence (Table 1, entries 3 and 4) of ethyldiisopropylamine.^[30] When the strain on the pyranose ring was released by changing the 4,5-*O*-protecting groups from a cyclic (isopropylidene) to an acyclic (TBS) group, we observed a decrease in the yields of the coupling products, but also a significant suppression of the formation of the undesired glycals (Table 1, entries 1,2 and 3,4). These protecting groups apparently control the conformation of the pyranose ring and affect the stability of the



Scheme 3. Synthesis of Kdo donors α -**3** and α/β -**5**. a) DAST, CH_2Cl_2 , MS4A, $-78 \rightarrow 25^\circ\text{C}$, 30 min (66% for α -**3**, 95% for α/β -**5**); b) $[\text{Pd}_2(\text{dba})_3]$, $\text{AllylOCO}_2\text{Et}$, dppb, THF, 65°C , 2 h (89%); c) aqueous AcOH (90%), reflux, 1 h (92%); d) TBSOTf, 2,6-lutidine, CH_2Cl_2 , 15 h (96%); e) $[\text{Ir}(\text{H}_2)(\text{cod})(\text{MePh}_2\text{P})_2]\text{PF}_6$, THF, 1 h; f) I_2 , H_2O , 20 min (85%). dba = dibenzylideneacetone, dppb = 1,4-bis(diphenylphosphanyl)butane, Tf = trifluoromethanesulfonyl, cod = 1,5-cyclooctadiene.



Scheme 4. Glycosylation of glucosamine acceptors **16** and **17** with Kdo donors α -**3** and α/β -**5**. [a] Contaminated by the glycal **13** which is, however, inert during the reaction. [b] Used as a mixture of anomers (α/β = 4:1). [c] R^4COOH = (*R*)-3-(benzyloxy)tetradecanoic acid.

Table 1. Glycosylation of glucosamine acceptors **16** and **17** with Kdo donors α -**3** and α/β -**5** (see Scheme 4).^[a]

Entry	Donor (equiv)	Acceptor	$\text{BF}_3 \cdot \text{OEt}_2$ [equiv]	$i\text{Pr}_2\text{NEt}$ [equiv]	Disaccharide (yield [%], α/β)	Glycal (yield [%])
1	α - 3 (4.0)	16	2.5	2.0	18 (72, 90:10)	13 (61)
2	α/β - 5 (4.0)	16	2.5	2.0	19 (45, 94:6)	15 (10)
3	α - 3 (2.0)	17	1.1	–	18 (83, 87:13)	13 (42)
4	α/β - 5 (2.0)	17	1.5	–	19 (81, 93:7)	15 (5)

[a] Yields of disaccharides and glycals are based on glycosyl acceptors and glycosyl donors, respectively.

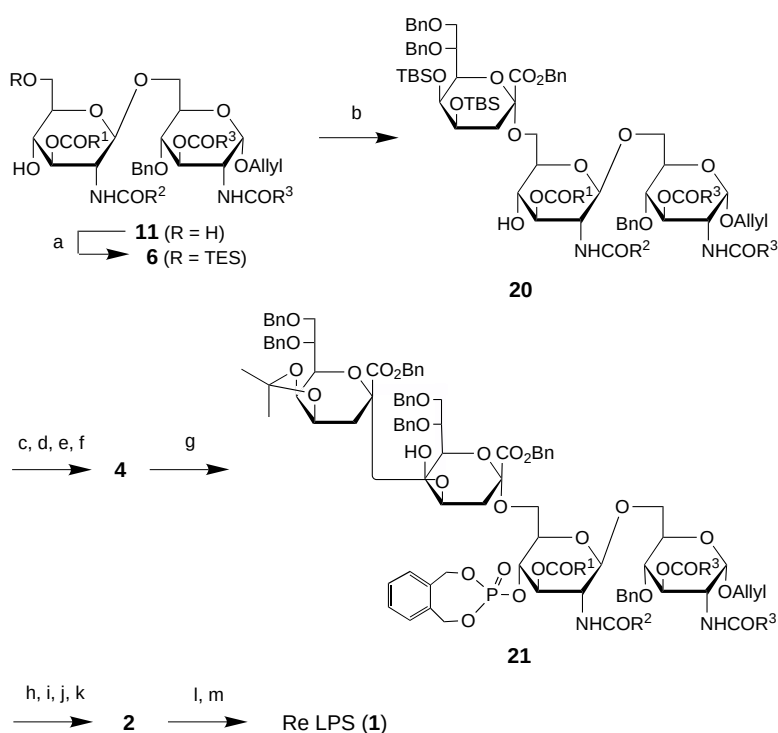
oxocarbenium ionic intermediates generated from the corresponding Kdo fluorides. Indeed, the glycosylation of **16** with 2.5 equivalents of a 4,5-*O*-tetraisopropylidisiloxanylidene-(TIPDS)-protected Kdo fluoride (data and structure not shown) afforded the corresponding disaccharide and the glycal in yields of 50% (based on **16**) and 42% (based on the fluoride), respectively. Both values lie between the respective values of the isopropylidene- and TBS-protected derivatives (Table 1, entries 1 and 2), and correspond to the order of the conformational flexibility of the pyranose ring: isopropylidene < TIPDS < TBS. All the reactions in Table 1 were highly α -selective, irrespective of the anomeric stereochemistry of the donors employed. Higher α -stereoselectivities were observed in the glycosylation with the TBS-protected Kdo fluoride **5** (Table 1, entries 2 and 4). Triethylsilylation of the acceptor greatly improved the yields, even when fewer equivalents of the Kdo donors were used (Table 1, entries 3 and 4). Based on these results, the TBS-protected Kdo fluoride α/β -**5** and the triethylsilyl(TES)-protected glycosyl acceptor are the best coupling partners that provide Kdo glycosides in high yields and with high stereoselectivity.

The final stages that lead to the completion of our synthesis of Re LPS are shown in Scheme 5. TES ether **6** was prepared by the selective protection of diol **11**, and then coupled with the TBS-protected Kdo fluoride α/β -**5**. The desired $\alpha(2 \rightarrow 6)$ -

glycoside **20** was obtained with complete regio- and stereoselectivity, which was unambiguously confirmed by ^1H NMR spectroscopy (Table 2). The free 4'-hydroxy group of **20** was phosphorylated to give the protected 4'-phosphate,^[31] which was then subjected to desilylation followed by regioselective triethylsilylation to give **4**, ready for the incorporation of the second Kdo residue. The use of α/β -**5** also as the second Kdo source gave the desired tetrasaccharide in a disappointingly low yield of 26%, probably due to the steric repulsion between both coupling components. In fact, when the less bulky isopropylidene-protected Kdo fluoride α -**3** was employed—an excess (4 equiv) was used to compensate for possible side reactions resulting from its higher activity—the desired tetrasaccharide **21** was obtained in 75% yield with complete regio- and stereoselectivity, which was confirmed by detailed ^1H NMR spectroscopic analysis (Table 2). After the removal of the isopropylidene and allyl groups, the resulting anomeric hydroxy group was selectively phosphorylated^[32] to give the protected Re LPS derivative **2**. The removal of the isopropylidene group of **21** by aqueous acetic acid at 80 °C unexpectedly also resulted in interresidual lactonization of Kdo (26%). This side reaction was almost completely diminished by the use of aqueous trifluoroacetic acid (TFA) in CH_2Cl_2 at 0 °C for the deisopropylidenation. Finally, all the benzyl-type protecting groups were removed by hydrogenolysis. The lactone and/or 1-*O*-dephosphorylated derivatives

formed during this reaction were effectively removed by liquid–liquid partition column chromatography using a two-layered solvent system (*n*BuOH/THF/H₂O/MeOH 16/7/1/20) on Sephadex LH-20 gel to give the first synthetic, highly pure Re LPS.^[33] The product was identical to natural Re LPS (NMR spectroscopy,^[34] MS, and behavior on TLC (Table 2)), except for the presence of several minor peaks or spots (data not shown) that were present in the natural specimen, even after purification (see below).

The synthetic Re LPS exhibited the same level of cytokine(TNF- α and IL-6)-inducing activity in heparinized human peripheral whole blood cells^[35] as did natural Re LPS that had been purified by liquid–liquid partition column chromatography in the same manner as described above. The amounts of TNF- α induced by synthetic and natural specimens (100 ng mL⁻¹) were found to be 871 and 1047 ng mL⁻¹, respectively. The level of IL-6 (4760 ng mL⁻¹) induced by the same natural Re LPS (10 ng mL⁻¹) was also higher than that (3331 ng mL⁻¹) induced by the same dose of the synthetic Re LPS. The slightly higher activity of natural Re LPS can be explained by the presence of more highly active contaminants, most likely those containing longer saccharide chains. These minor contaminants are still detectable by spectroscopic and TLC analyses, even after extensive purification. This result clearly demonstrates the obvious merit of chemical synthesis, which completely excludes the influences of other bacterial components. Only by this



Scheme 5. Final steps leading to Re LPS (**1**). a) TESCl, imidazole, CH_2Cl_2 , 0 °C, 1 h (85%); b) α/β -**5**, $\text{BF}_3 \cdot \text{OEt}_2$, MS4A, CH_2Cl_2 , 0 °C, 1 h (89%); c) *N,N*-diethyl-1,5-dihydro-3*H*-2,3,4-benzodioxaphosphin-3-amine, 1*H*-tetrazole, MS4A, CH_2Cl_2 , 40 min; d) MCPBA, -20 °C, 1 h (96% over two steps); e) HF (47%)/ CH_2Cl_2 /MeCN (1/5/5), 5 h (89%); f) TESCl, imidazole, MS4A, CH_2Cl_2 , 2 h (85%); g) α -**3**, $\text{BF}_3 \cdot \text{OEt}_2$, MS4A, CH_2Cl_2 , 0 °C, 1 h (75%); h) TFA, H_2O , CH_2Cl_2 , 0 °C, 4 h (82%); i) $[\text{Ir}(\text{H}_2)\text{-(cod)}(\text{MePh}_2\text{P})_2]\text{PF}_6$, THF, 1 h; j) I_2 , H_2O (82%); k) tetrabenzyl diphosphate, $\text{LiN}(\text{TMS})_2$, THF, -78 $\rightarrow$ 20 °C, 2.5 h; l) **1** (10–20 kg cm⁻²), Pd (black), THF, 1 d; m) liquid–liquid partition column chromatography (see text) (22% over three steps). MCPBA = *meta*-chloroperoxybenzoic acid.

Table 2. Selected data for **20**, **21**, and synthetic **1**.

20: R_f = 0.50 (silica gel, $\text{CHCl}_3/\text{acetone}$ 20/1); ^1H NMR (500 MHz, CDCl_3 , 30 °C, TMS): δ = 7.57 (d, J = 8.2 Hz, 2H; CF_3Ph), 7.48 (d, J = 8.2 Hz, 2H; CF_3Ph), 7.42 (d, J = 8.0 Hz, 2H; CF_3Ph), 7.35–7.16 (m, 22H; CF_3Ph , Ph), 6.09 (d, J = 9.4 Hz, 1H; NH), 5.87 (d, J = 8.2 Hz, 1H; NH'), 5.55 (m, 1H; allyl), 5.28 and 5.14 (AB, J = 12.2 Hz, 2H; ArCH_2), 5.27 (t, J = 9.8 Hz, 1H; H-3), 5.20 (m, 1H; acyl), 5.06–4.99 (m, 3H; H-3', allyl, acyl), 4.97 (dd, J = 10.5, 1.4 Hz, 1H; allyl), 4.63 (d, J = 3.5 Hz, 1H; H-1), 4.60–4.52 (m, 7H; H-1', ArCH_2), 4.45 and 4.43 (AB, J = 11.4 Hz, 2H; ArCH_2), 4.45 (AB, J = 11.4 Hz, 1H; ArCH_2), 4.42 (AB, J = 12.4 Hz, 1H; ArCH_2), 4.22 (ddd, J = 12.9, 10.6, 3.5 Hz, 1H; H-2), 4.12 (brs, 1H; H-5''), 4.06 (m, 1H; H-4''), 3.97–3.79 (m, 8H; H-6a, H-6a', H-6'', H-7'', H-8a'', allyl, acyl), 3.76–3.59 (m, 5H; H-4, H-5, H-6b, H-2', H-8b''), 3.55 (dd, J = 10.3, 6.4 Hz, 1H; H-6b'), 3.49–3.39 (m, 3H; H-4', H-5', allyl), 2.62 (dd, J = 15.6, 7.8 Hz, 1H; acyl), 2.57–2.50 (m, 2H; acyl), 2.39 (dd, J = 16.3, 4.8 Hz, 1H; acyl), 2.34–2.22 (m, 7H; acyl), 2.28–2.11 (m, 2H; H-3a'', acyl), 1.76 (dd, J = 12.4, 4.3 Hz, 1H; H-3b''), 1.59–1.23 (m, 120H; acyl), 0.89–0.82 (m, 36H; acyl, $t\text{BuSi}$), 0.07 (s, 3H; MeSi), 0.06 (s, 3H; MeSi), 0.04 (s, 3H; MeSi), 0.02 (s, 3H; MeSi); ESI-MS (positive-ion mode): m/z : calcd for $\text{C}_{161}\text{H}_{254}\text{F}_6\text{N}_2\text{O}_{26}\text{Si}_2\text{Na}$ [$M+\text{Na}$] $^+$: 2824.8, found 2824.9

21: R_f = 0.54 (silica gel, toluene/EtOAc 4/1); ^1H NMR (500 MHz, CDCl_3 , 30 °C, TMS): δ = 7.55 (d, J = 8.1 Hz, 2H; CF_3Ph), 7.48 (d, J = 8.0 Hz, 2H; CF_3Ph), 7.49 (d, J = 8.0 Hz, 2H; CF_3Ph), 7.34–7.11 (m, 40H; CF_3Ph , Ph, $o\text{-C}_6\text{H}_4(\text{CH}_2\text{O})_2\text{P}$), 6.86 (d, J = 7.2 Hz, 1H; $o\text{-C}_6\text{H}_4(\text{CH}_2\text{O})_2\text{P}$), 6.12 (d, J = 9.3 Hz, 1H; NH), 6.10 (d, J = 9.4 Hz, 1H; NH'), 5.57 (m, 1H; allyl), 5.50 (dd, J = 10.2, 10.0 Hz, 1H; H-3'), 5.31 (dd, J = 10.8, 9.3 Hz, 1H; H-3), 5.26 (m, 1H; acyl), 5.12–4.82 (m, 13H; H-1', allyl, acyl, ArCH_2 , $o\text{-C}_6\text{H}_4(\text{CH}_2\text{O})_2\text{P}$), 4.70 and 4.65 (AB, J = 11.4 Hz, 2H; ArCH_2), 4.66 (d, J = 3.5 Hz, 1H; H-1), 4.59–4.34 (m, 16H; H-4', H-4'', H-4''', H-5'', ArCH_2), 4.22 (ddd, J = 10.8, 9.3, 3.5 Hz, 1H; H-2), 4.12 (d, J = 9.0 Hz, 1H; H-6a), 4.01 (m, 1H; H-7''), 3.96–3.90 (m, 3H; H-5'', H-6'', allyl), 3.86–3.84 (m, 2H; H-6'', H-8a''), 3.81–3.74 (m, 7H; H-6a', H-8a'', H-8b'', H-7'', H-8b''', acyl), 3.72–3.61 (m, 6H; H-4, H-5, H-6b, H-5', H-6b', allyl), 3.34 (ddd, J = 10.1, 8.0, 7.0 Hz, 1H; H-2'), 2.88 (dd, J = 15.3, 3.4 Hz, 1H; H-3a''), 2.61 (d, J = 6.6 Hz, 2H; acyl), 2.54 (brs, 1H; 5'-OH), 2.47 (dd, J = 16.3, 7.1 Hz, 1H; acyl), 2.37–2.22 (m, 9H; H-3a'', acyl), 2.17 (dd, J = 15.2, 5.3 Hz, 1H; acyl), 2.07 (dd, J = 12.7, 12.0 Hz, 1H; H-3b''), 1.92 (dd, J = 15.3, 2.6 Hz, 1H; H-3b'''), 1.63–1.19 (m, 126H; isopropylidene, acyl), 0.89–0.85 (m, 18H; acyl); ESI-MS (positive-ion mode): m/z : calcd for $\text{C}_{189}\text{H}_{267}\text{F}_6\text{N}_2\text{O}_{36}\text{PNa}$ [$M+\text{Na}$] $^+$: 3308.9, found 3308.8

Synthetic Re LPS (**1**): R_f = 0.29 (silica gel, $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}/\text{Et}_3\text{N}$ 60/40/10/0.02); ^1H NMR (500 MHz, Triton X-100/ D_2O , 30 °C, $\delta(\text{HDO})$ as reference): δ = 5.29 (m, 1H; H-1), 5.15 (m, 1H; acyl), 5.13 (m, 1H; H-3), 5.06 (m, 1H; acyl), 5.06 (m, 1H; H-3'), 4.53 (m, 1H; H-4'), 4.52 (m, 1H; H-1'), 4.03–3.32 (m, 16H; H-4, H-5, H-6a, H-6b, H-5', H-6a', H-6b', H-5'', H-6'', H-7'', H-8a'', H-8b'', H-6'', H-7'', H-8a'', H-8b'', 4.01 (m, 1H; H-2), 3.98 (m, 1H; H-4'''), 3.90 (m, 1H; H-4''), 3.87 (m, 1H; acyl), 3.80 (m, 1H; acyl), 3.76 (m, 1H; H-2'), 3.73 (m, 1H; H-5''), 2.56 (m, 2H; acyl), 2.44 (m, 2H; acyl), 2.33 (m, 3H; acyl), 2.22 (m, 5H; acyl), 1.99 (m, 1H; H-3a''), 1.84 (m, 1H; H-3a'''), 1.79 (m, 1H; H-3b'''), 1.63 (m, 1H; H-3b''), 1.60–1.20 (m, 120H; acyl), 0.71 (m, 18H; acyl); ESI-MS (negative-ion mode): m/z : calcd for $\text{C}_{110}\text{H}_{200}\text{N}_2\text{O}_{39}\text{P}_2$ [$M-2\text{H}$] $^{2-}$: 1117.7, found 1117.8

approach can the true biological activity be discussed precisely in relation to chemical structures.

In summary, we have accomplished the first total synthesis of Re LPS starting from D-glucosamine hydrochloride with an overall yield of 0.9% over 23 steps. In combination with an efficient purification method based on liquid–liquid partition, structural congeners such as the one lacking the terminal Kdo residue (Kdo-lipid A) and its 4'-O-dephosphorylated analogue have also been successfully synthesized by using a similar synthetic pathway.^[36] The present work has opened a new stage of research into bacterial LPS at precise molecular levels, for example, its recognition by receptors and detailed analyses of the relationships between bioactivity and three-

dimensional molecular structures, which are currently under intensive investigation in our laboratory.

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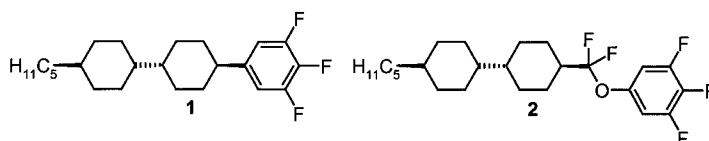
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Diffuorooxymethylene-Bridged Liquid Crystals: A Novel Synthesis Based on the Oxidative Alkoxydifluorodesulfuration of Dithianylum Salts**

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Dedicated to Professor Heinz A. Staab on the occasion of his 75th birthday

In our search for new, superior liquid crystals^[1] for use in active-matrix liquid crystal displays^[2] (AM-LCD or thin film transistor LCD, TFT-LCD) it was found that the insertion of a difluorooxymethylene bridge into a specific location of the mesogenic core structure of phenylbicyclohexyl-type liquid crystals (**1**)^[3] results in a class of materials (**2**; Scheme 1)^[4] that



Scheme 1. The liquid crystalline basic structure **1** and its analogue **2** which is extended by a difluorooxymethylene bridge.

exhibits a surprising improvement of essentially all application-relevant properties (see Table 1). These include a broader nematic phase range, a higher dielectric anisotropy ($\Delta\epsilon$), a lower rotational viscosity (γ_1)^[5] but also a higher specific resistivity and voltage holding ratio.^[6] Since the liquid crystals **1** are currently the most commonly used type of materials for all kinds of AM-LCDs, this fundamental improvement represents significant progress for LCD technology as a whole with regard to faster switching times and a broader operating temperature range.

Whilst there is a variety of methods reported for the preparation of aryl α,α -difluorobenzyl ethers,^[7] the efficient synthesis of aryl and alkyl α,α -difluoroalkyl ethers has been an unsolved problem so far. The first small amounts of **2**^[4b, 8] and its analogues were synthesized by the general methods depicted in Scheme 2. But both synthetic routes suffer from serious drawbacks, such as low yields and difficult purification of the intermediates **7**^[4b, 8] and **8**,^[9–11] and of the target compounds **9**. Therefore, it was our aim to develop a new,

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